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WASHINGTON, D.C. 20460

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

AUG 2 1997

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: DICAMBA: Review of Mutagenicity Studies with the Dimethylamine (DMA), Diglycolamine (DGA) and Isopropylamine (IPA) Salts of Dicamba.

FROM: Jess Rowland, M.S., Branch Senior Scientist *Jess Rowland* 6/27/97
Science Analysis Branch, Health Effects Division (7509C)

TO: Walter Waldrop / Jane Mitchell
Product Manager 71
Reregistration Division (7508W)

THRU: Alberto Protzel, Ph.D., Branch Senior Scientist
Toxicology Branch I, Health Effects Division (7509C)

Alberto Protzel 7/29/97

DATA PACKAGE

IDENTIFICATION: Submission: S473825

DP Barcode: D207648

Chemical	PC Code	Tox.Chem.No.	MRID No(s).
Dicamba-DMA	029802	295B	43354332
Dicamba-DGA	128931	295F	43354333
Dicamba-IPA	128944	295G	43354334

ACTION REQUESTED: Review the *in vivo* micronucleus assays in mice with the DMA, DGA and IPA salts of dicamba submitted by Sandoz Inc, to fulfill Subdivision F guideline requirements §84-2.

RESPONSE: Data Evaluation Records (DERs) for the three studies referenced above are attached. The Executive Summaries are presented below. The DMA, DGA and IPA salts of dicamba were shown to be non mutagenic in *in vivo* micronucleus assays in mice,.

These studies are classified as **Acceptable/Guideline** and satisfies the Subdivision F guideline requirement (§84-2a,b) for *in vitro* mutagenicity assays.



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I. DIMETHYLAMINE Salt of Dicamba

CITATION: Putman, D., and R. Young. (1994) Micronucleus cytogenetic assay in mice. Microbiological Associates, Inc., Rockville, MD. Laboratory Study Number TE236.122. 8/5/94. **MRID No. 43354332.** Unpublished.

EXECUTIVE SUMMARY: In an *in vivo* mouse bone marrow micronucleus assay (MRID No. 43354332), groups of five male and five female ICR mice received a single IP injection of 450, 900, or 1,800 mg/kg of the DMA salt of dicamba (40.3% ai). Bone marrow cells were harvested at 24, 48, and 72 hours posttreatment and scored for micronucleated polychromatic erythrocytes (MPCEs). Mortality occurred in 4/20 male and 3/20 female mice dosed at 1,800 mg/kg and in 1/15 males dosed at 900 mg/kg. Lethargy was observed in male and female mice at all dose levels. The DMA salt of dicamba was not cytotoxic to the target cell. The positive control induced significant increases in MPCEs in both sexes. **The DMA salt of dicamba was non-mutagenic. There was no significant increase in the frequency of MPCEs in bone marrow after any treatment time.**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for *in vivo* cytogenetic mutagenicity data (§84-2).

II. DIGLYCOLAMINE Salt of Dicamba (MRID No. 43354333)

CITATION: Putman, D., and R. Young. (1994) Micronucleus cytogenetic assay in mice. Microbiological Associates, Bethesda, MD. Laboratory Study Number TE237.122. 8/5/94. **MRID No. 43354333.** Unpublished.

EXECUTIVE SUMMARY: In an *in vivo* mouse bone marrow micronucleus assay (MRID No. 43354333), groups of five ICR mice/sex received a single IP injection of 525, 1050, or 2100 mg/kg of the DGA salt formulation of dicamba (39.7% ai). Bone marrow cells were harvested at 24, 48, or 72 hours posttreatment and scored for micronucleated polychromatic erythrocytes (MPCEs). Mortality occurred in 3/20 male and 1/20 female mice dosed at 2100 mg/kg. Lethargy was observed in male and female mice at all dose levels. Cytotoxicity by the DGA salt formulation was observed by a reduction in the ratio of PCEs to total erythrocytes in males dosed at 2100 mg/kg 48 and 72 hours following dosing. The positive control induced significant increases in MPCEs in both sexes. **The DGA salt of dicamba was non-mutagenic. There was no significant increase in the frequency of MPCEs in bone marrow after any treatment time.**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for *in vivo* cytogenetic mutagenicity data (§84-2).

III. ISOPROPYLAMINE Salt of Dicamba

CITATION: Putman, D., and R. Young. (1994) Micronucleus cytogenetic assay in mice. Microbiological Associates, Bethesda, MD. Laboratory Study Number TE238.122. 8/5/94. **MRID** No. 43354334. Unpublished.

EXECUTIVE SUMMARY: In an *in vivo* mouse bone marrow micronucleus assay (MRID No. 43354334), groups of five ICR mice/sex received a single IP injection of 500, 1000, or 2000 mg/kg of the IPA salt formulation of dicamba (32.3% ai). Bone marrow cells were harvested at 24, 48, or 72 hours posttreatment and scored for micronucleated polychromatic erythrocytes (MPCEs). Mortality occurred in 2/20 male and 0/20 female mice dosed at 2000 mg/kg. Lethargy was observed in male and female mice at all dose levels. The IPA salt formulation of dicamba was not cytotoxic to the target cell. The positive control induced significant increases in MPCEs in both sexes. **The IPA salt of dicamba was non-mutagenic. There was no significant increase in the frequency of MPCEs in bone marrow after any treatment time.**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for *in vivo* cytogenetic mutagenicity data (§84-2).

DATA EVALUATION RECORD**DICAMBA AMINE SALTS**

Study Type: 84-2; Micronucleus Assay in Mice (IPA Salt)

Work Assignment No. 1-14C (MRID 43354334)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
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Primary Reviewer:
Mary Menetrez, Ph.D.

Signature: Mary Menetrez
Date: 4/17/96

Secondary Reviewer:
Steven Brecher, Ph.D.

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Date: 4/15/96

Project Manager:
William Spangler, Ph.D.

Signature: William J. Spangler
Date: 4/8/96

Quality Assurance:
Reto Engler, Ph.D.

Signature: Reto Engler
Date: 4/8/96

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

DICAMBA AMINE SALTS

Micronucleus Assay (84-2)

EPA Reviewer: Jess Rowland, M.S. *Jess Rowland 6/20/97*
Branch Senior Scientist, Science Analysis Branch

EPA Secondary Reviewer: Alberto Protzel, Ph.D. *Alberto Protzel 7/27/97*
Branch Senior Scientist, Toxicology Branch I

DATA EVALUATION RECORD

STUDY TYPE: *In vivo* mammalian cytogenetics - micronucleus assay in mice

OPP Guideline Number: §84-2

DP BARCODE: D207648

SUBMISSION CODE: None

P.C. CODE: 128944

TOX. CHEM. NO.: 295G

TEST MATERIAL (PURITY): Isopropylamine (IPA) salt of dicamba (32.3% ai)

SYNONYMS: IPA salt of dicamba

CITATION: Putman, D., and R. Young. (1994) Micronucleus cytogenetic assay in mice. Microbiological Associates, Bethesda, MD. Laboratory Study Number TE238.122. 8/5/94. **MRID No. 43354334**. Unpublished.

SPONSOR: Sandoz Agro, Inc., Des Plaines, IL.

EXECUTIVE SUMMARY: In an *in vivo* mouse bone marrow micronucleus assay (MRID No. 43354334), groups of five ICR mice/sex received a single IP injection of 500, 1000, or 2000 mg/kg of the IPA salt formulation of dicamba (32.3% ai). Bone marrow cells were harvested at 24, 48, or 72 hours posttreatment and scored for micronucleated polychromatic erythrocytes (MPCEs).

Mortality occurred in 2/20 male and 0/20 female mice dosed at 2000 mg/kg. Lethargy was observed in male and female mice at all dose levels. The IPA salt formulation of dicamba was not cytotoxic to the target cell. The positive control induced significant increases in MPCEs in both sexes. **The IPA salt of dicamba was non-mutagenic. There was no significant increase in the frequency of MPCEs in bone marrow after any treatment time.**

This study is classified as **Acceptable**/Guideline and satisfies the guideline requirements for *in vivo* cytogenetic mutagenicity data (§84-2).

I. MATERIALS AND METHODS**A. MATERIALS****1. Test Material:** IPA salt of dicamba

Description: Caramel-colored viscous liquid

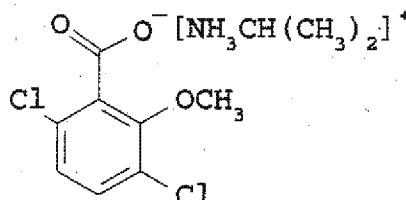
Lot/Batch #: 5998-3

Purity: 32.3% ai

Stability of compound: Not reported

CAS #: 55871-02-8

Structure:



Solvent used: Deionized distilled water

Other comments: The test material was stored at room temperature and protected from light.

2. Control Materials

Vehicle/Final volume/Route of administration: Deionized distilled water, 10 mL/kg, IP injection

Positive/Final dose/Route of administration: Cyclophosphamide (CP) in deionized distilled water; 40 mg/kg

3. Test compound administration

Volume of test substance administered: 10 mL/kg

Route of administration: IP injection

Dose levels used:

Pilot Study: 1, 10, 100, 1000, 5000 mg/kg

Toxicity Study: 1400, 2000, 2700, 3800 mg/kg

Micronucleus Assay: 500, 1000, 2000 mg/kg

Rationale for dose selection: The high dose of 2000 mg/kg was approximately 80% of the LD_{50/3} determined from the toxicity study.

DICAMBA AMINE SALTS

Micronucleus Assay (84-2)

4. Test animals

a. Species: Mouse (Strain ICR)

Age: 6-8 weeks

Weight: Pilot Study, male 29.1-35.2 g, female 22.2-25.6 g;

Toxicity Study, male 29.5-34.9 g, female 22.6-25.6 g;

Micronucleus Assay, male 29.5-36.6 g, female 25.5-32.0 g

Source: Harlan Sprague Dawley, Inc., Frederick, MD.

b. No. animals used per dose:

Pilot Study: 5/sex at 5,000 mg/kg, and 2 males/dose at 1, 10, 100, 1,000 mg/kg

Toxicity Study: 5/sex/dose

Micronucleus Assay: 15/sex/dose, plus 5/sex as replacement animals at the high dose. An additional 5/sex received the positive control substance.

c. Properly maintained? Yes

B. TEST PERFORMANCE1. Treatment and Sampling Times

a. Test compound and vehicle control

Dosing: Once

Sampling: 24, 48, and 72 hours after dosing

b. Positive control:

Dosing: Once

Sampling: 24, 48, and 72 hours after dosing

2. Tissues and Cells Examined

Bone marrow was the only tissue examined.

No. of polychromatic erythrocytes (PCEs) examined per animal: 1,000No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal: 1,000 erythrocytes were counted and the proportion of PCEs to total erythrocytes was calculated.3. Details of slide preparation

At 24, 48, and 72 hours after dosing, animals from each dose group were sacrificed by CO₂ asphyxiation. Marrow was aspirated from the femur and mixed with fetal bovine serum. After centrifugation and resuspension, cells were spread on slides, fixed in methanol, stained with May-Grunwald-Giemsa, and permanently mounted. Slides were coded prior to scoring.

4. Statistical methods

The incidence of MPCEs per 1,000 PCEs was determined for each animal and treatment group. Statistical significance ($p \leq 0.05$) of the incidence of MPCEs was determined using Kastenbaum-Bowman tables.

5. Evaluation Criteria

The test was considered valid if the number of MPCEs in the negative (vehicle) control did not exceed 5/1,000 PCEs and if the incidence of MPCEs in the positive control significantly increased with respect to the negative control ($p \leq 0.05$).

A positive response was a dose-responsive increase in the MPCEs with one or more dose levels statistically elevated relative to the vehicle control ($p \leq 0.05$, Kastenbaum-Bowman tables).

- C. COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

II. REPORTED RESULTS

A. Solubility/Analytical Determinations

The test material was soluble in water. Dosing solutions were prepared on the days of testing and samples were analyzed by HPLC to confirm the nominal concentrations. The dosing solutions were 96.6-106% of the nominal concentrations.

B. Pilot study

In a pilot study, five mice/sex were administered the IPA salt formulation of dicamba by IP injection at 5000 mg/kg and to two male mice each at 1, 10, 100, or 1000 mg/kg. Mortality occurred in 5/5 males and 5/5 females dosed at 5000 mg/kg. Within 1 hour of dosing, lethargy was observed in mice dosed at 1000 mg/kg. The mice dosed at 1000 mg/kg appeared normal within 48 hours of dosing and other animals dosed at ≤ 100 mg/kg appeared normal throughout the observation period.

C. Toxicity Study

Groups of five mice/sex were dosed with the IPA salt formulation of dicamba by IP injection at 1400, 2000, 2700, or 3800 mg/kg. Body weight and mortality data for the toxicity study are presented in Appendix 1 (Table 1; study report page 14) included in this DER. Mortality occurred within 3 days in all animals dosed at 3800 mg/kg, in 3/5 males and 2/5 females dosed at 2700 mg/kg, and in 1/5 males dosed at 2000 mg/kg.

DICAMBA AMINE SALTS

Micronucleus Assay (84-2)

There were no deaths in animals dosed at 1400 mg/kg or females dosed at 2000 mg/kg. Clinical signs, first noted on the day of dose administration, included lethargy. $LD_{50/3}$ was calculated by probit analysis to be approximately 2481 mg/kg. Based on these results, a high dose of 2000 mg/kg (approximately 80% of the $LD_{50/3}$) was chosen for the micronucleus assay.

D. Micronucleus Assay

Separate studies were performed for male and female mice.

1. Animal observations: Mortality was observed in 2/20 male and 0/20 female mice dosed at 2000 mg/kg. Lethargy was observed in male and female mice at all dose levels.
2. Micronucleus assay: The results of the bone marrow micronucleus assay are presented in Appendix 2 (Table 2; study report page 15) included in this DER. The IPA salt formulation of dicamba was neither cytotoxic to the target organ nor caused a statistically significant increase in MPCEs, compared to vehicle controls, in bone marrow cells collected from male or female mice 24, 48, or 72 hours after dosing at 500, 1000, or 2000 mg/kg. The positive control (40 mg/kg cyclophosphamide) induced significant ($p \leq 0.05$) increases in MPCEs in both sexes.

The study authors concluded that the IPA salt formulation of dicamba was negative in this *in vivo* mouse micronucleus assay.

III. DISCUSSION/CONCLUSIONS

A. Investigator's Conclusions

The study authors concluded that the IPA salt formulation of dicamba did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in mouse bone marrow, and was negative in the micronucleus test using male and female ICR mice.

B. Reviewer's Discussion

The reviewer agrees with the study authors that the IPA salt formulation of dicamba was not clastogenic or aneugenic in this *in vivo* assay when tested to a dose level of 2000 mg/kg. The sensitivity of this test to detect genotoxic response was demonstrated by the significant ($p \leq 0.05$) increase in MPCEs induced by the positive control (40 mg/kg CP). We conclude that the IPA salt of dicamba was adequately tested and found non-genotoxic in this *in vivo* micronucleus assay.

IV. STUDY DEFICIENCIES

None.

1. [Illegible text]

APPENDIX 1

TABLE 1
TOXICITY STUDY WITH IPA SALT OF DICAMBA IN ICR MICE
BODY WEIGHT AND MORTALITY DATA

TREATMENT	SEX	GROUP MEAN BODY WEIGHTS (gms)			% CHANGE ¹		MORTALITY ²
		PRETREATMENT	DAY 1	DAY 3	DAY 1	DAY 3	
IPA Salt of Dicamba 1400 mg/kg	M	31.2 ± 1.8	30.9 ± 1.4	32.0 ± 1.8	-1.0%	2.6%	0 / 5
	F	23.1 ± 1.6	23.0 ± 1.0	24.2 ± 1.3	-0.4%	4.8%	0 / 5
2000 mg/kg	M	28.8 ± 2.6	30.0 ± 2.7	32.6 ± 1.9	4.2%	13.2%	1 / 5
	F	22.9 ± 1.0	22.6 ± 1.4	23.1 ± 1.8	-1.3%	0.9%	0 / 5
2700 mg/kg	M	31.7 ± 2.9	30.9 ± 2.8	31.9 ± 2.8	-2.5%	0.6%	3 / 5
	F	23.8 ± 1.2	23.3 ± 1.3	23.5 ± 1.9	-2.1%	-1.3%	2 / 5
3800 mg/kg	M	32.0 ± 1.4	NA ³	NA	NA	NA	5 / 5
	F	24.1 ± 0.9	NA	NA	NA	NA	5 / 5

¹% Change = $\frac{(\text{Post-treatment weight} - \text{Pretreatment weight}) \times 100}{\text{Pretreatment weight}}$

²Reported as number of animals dead 3 days after dose administration/total number tested.

³NA = No data due to mortality.

APPENDIX 2

TABLE 2

SUMMARY OF BONE MARROW MICRONUCLEUS STUDY WITH IPA SALT OF DICAMBA IN ICR MICE

TREATMENT	SEX	TIME (HR)	NUMBER OF MICE	PCE/TOTAL ERYTHROCYTES	MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES	
					NUMBER PER 1000 PCE'S (MEAN \pm S.D.)	NUMBER PER PCE'S SCORED ¹
Water 10 ml/kg	M	24	5	0.64	0.8 \pm 1.79	4 / 5000
		48	5	0.57	1.2 \pm 0.84	6 / 5000
		72	5	0.61	0.0 \pm 0.00	0 / 5000
	F	24	5	0.57	0.4 \pm 0.55	2 / 5000
		48	5	0.64	0.6 \pm 0.89	3 / 5000
		72	5	0.48	0.0 \pm 0.00	0 / 5000
IPA Salt of Dicamba 500 mg/kg	M	24	5	0.69	0.4 \pm 0.55	2 / 5000
		48	5	0.57	0.4 \pm 0.55	2 / 5000
		72	5	0.59	0.4 \pm 0.55	2 / 5000
	F	24	5	0.56	0.6 \pm 1.34	3 / 5000
		48	5	0.63	0.8 \pm 1.30	4 / 5000
		72	5	0.57	0.2 \pm 0.45	1 / 5000
1000 mg/kg	M	24	5	0.68	1.2 \pm 1.10	6 / 5000
		48	5	0.48	0.4 \pm 0.89	2 / 5000
		72	5	0.57	0.6 \pm 0.55	3 / 5000
	F	24	5	0.54	0.8 \pm 1.30	4 / 5000
		48	5	0.67	0.4 \pm 0.89	2 / 5000
		72	5	0.59	0.2 \pm 0.45	1 / 5000
2000 mg/kg	M	24	5	0.60	0.4 \pm 0.55	2 / 5000
		48	5	0.58	0.4 \pm 0.89	2 / 5000
		72	5	0.65	0.6 \pm 0.89	3 / 5000
	F	24	5	0.60	0.6 \pm 0.89	3 / 5000
		48	5	0.64	0.4 \pm 0.89	2 / 5000
		72	5	0.62	0.0 \pm 0.00	0 / 5000
CP, 40 mg/kg	M	24	5	0.41	9.4 \pm 6.91	47 / 5000*
	F	24	5	0.54	16.8 \pm 5.26	84 / 5000*

¹*, $p \leq 0.05$ (Kastenbaum-Bowman Tables)



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DICAMBA AMINE SALTS

Micronucleus Assay (84-2)

EPA Reviewer: Jess Rowland, M.S. *Jess Rowland 6/19/97*
Branch Senior Scientist, Science Analysis Branch

EPA Secondary Reviewer: Alberto Protzel, Ph.D. *Alberto Protzel 7/29/97*
Branch Senior Scientist, Toxicology Branch I

DATA EVALUATION RECORD

STUDY TYPE: *In vivo* mammalian cytogenetics - micronucleus assay in mice

OPP Guideline Number: §84-2

DP BARCODE: D207648

SUBMISSION CODE: S473825

P.C. CODE: 029802

TOX. CHEM. NO.: 295B

TEST MATERIAL (PURITY): Dimethylamine (DMA) salt of Dicamba (40.3% ai)

SYNONYMS: DMA salt of dicamba, Banvel

CITATION: Putman, D., and R. Young. (1994) Micronucleus cytogenetic assay in mice. Microbiological Associates, Inc., Rockville, MD. Laboratory Study Number TE236.122. 8/5/94. **MRID 43354332**. Unpublished.

SPONSOR: Sandoz Agro, Inc., Des Plaines, IL.

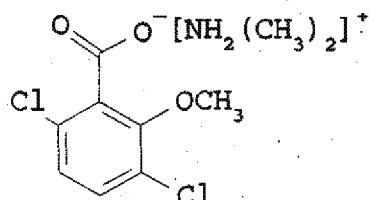
EXECUTIVE SUMMARY: In an *in vivo* mouse bone marrow micronucleus assay (MRID No. 43354332), groups of five male and five female ICR mice received a single IP injection of 450, 900, or 1,800 mg/kg of the DMA salt of dicamba (40.3% ai). Bone marrow cells were harvested at 24, 48, and 72 hours posttreatment and scored for micronucleated polychromatic erythrocytes (MPCEs).

Mortality occurred in 4/20 male and 3/20 female mice dosed at 1,800 mg/kg and in 1/15 males dosed at 900 mg/kg. Lethargy was observed in male and female mice at all dose levels. The DMA salt of dicamba was not cytotoxic to the target cell. The positive control induced significant increases in MPCEs in both sexes. **The DMA salt of dicamba was non-mutagenic. There was no significant increase in the frequency of MPCEs in bone marrow after any treatment time.**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for *in vivo* cytogenetic mutagenicity data (§84-2).

I. MATERIALS AND METHODS**A. MATERIALS**

1. Test Material: DMA salt of dicamba
Description: Caramel-colored viscous liquid
Lot/Batch #: 5998-5
Purity: 40.3% ai
Stability of compound: Not reported
CAS #: 2300-66-5
Structure:



Solvent used: Deionized distilled water

Other comments: The test material was stored at room temperature, protected from light.

2. Control Materials

Vehicle/Final volume/Route of administration: Deionized, distilled water, 10 mL/kg

Positive/Final dose(s)/Route of administration: Cyclophosphamide in sterile deionized distilled water; 40 mg/kg

3. Test compound administration

Volume of test substance administered: 10 mL/kg

Route of administration: IP injection

Dose levels used:

Pilot Study: 1, 10, 100, 1,000, 5,000 mg/kg

Toxicity Study: 1,400, 2,000, 2,700, 3,800 mg/kg

Micronucleus Assay: 450, 900, 1,800 mg/kg

Rationale for dose selection: The high dose of 1,800 mg/kg was approximately 80% of the LD_{50/3} determined in the toxicity study.

DICAMBA AMINE SALTS

Micronucleus Assay (84-2)

4. Test animals

a. Species: Mouse (Strain ICR)

Age 6-8 weeks

Weight: Pilot Study, male 29.1-35.2 g, female 22.2-25.6 g; Toxicity
Study: male 30.7-34.8 g, female 21.9-26.4 g; Micronucleus Assay:
male 29.5-36.6 g, female 25.5-32.0 g

Source: Harlan Sprague Dawley, Inc., Frederick, MD

b. No. animals used per dose:

Pilot Study: 5/sex at 5,000 mg/kg, 2 males each for the 1,000, 100, 10,
and 1 mg/kg doses

Toxicity Study: 5/sex

Micronucleus Assay: 15/sex/dose, plus 5/sex as replacement animals at
the high dose; 5/sex were used for positive controls.

c. Properly maintained? Yes

B. TEST PERFORMANCE1. Treatment and Sampling Times

a. Test compound and vehicle control:

Dosing: Once

Sampling: 24, 48, and 72 hours after dosing

b. Positive control:

Dosing: Once

Sampling: 24 hours after dosing

2. Tissues and Cells Examined

Bone marrow was the only tissue examined.

No. of polychromatic erythrocytes (PCEs) examined per animal: 1,000

No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per
1,000 PCEs animal; 1,000 erythrocytes were counted and the proportion
of PCEs to total erythrocytes was calculated.

3. Details of slide preparation

At 24, 48, and 72 hours after dosing, animals from each dose group were
sacrificed by CO₂ asphyxiation. Marrow was aspirated from the femur and mixed
suspended in fetal bovine serum. After centrifugation and re-suspension, cells
were spread on slides, fixed in methanol, stained with May-Grunwald-Giemsa and
permanently mounted. Slides were coded prior to scoring.

4. Statistical methods

The incidence of MPCEs per 1,000 PCEs was determined for each animal and treatment group. Statistical significance ($p \leq 0.05$) of the incidence of MPCEs was determined using Kastenbaum-Bowman tables.

5. Evaluation Criteria

The test was considered valid if the number of micronucleated PCEs in the negative (vehicle) control did not exceed 5/1,000 PCEs and if the incidence of micronucleated PCEs in the positive control significantly increased with respect to the negative control ($p \leq 0.05$).

A positive response was a dose-responsive increase in the micronucleated PCEs with one or more dose levels statistically elevated relative to the vehicle control ($p \leq 0.05$, Kastenbaum-Bowman tables). A significant increase in one treatment group at one sacrifice time with no evidence of a dose response would be considered suspect, and the assay would be repeated.

- C. COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

II. REPORTED RESULTS

A. Solubility/Analytical Determinations

The test material was soluble in water. The dosing solutions were prepared on the days of testing and samples from the micronucleus test were analyzed by HPLC to confirm the nominal concentrations. The dosing solutions were 91-101% of the nominal concentrations.

B. Pilot study

In a pilot study, five male and five female mice were administered the DMA salt of dicamba by IP injection at 5,000 mg/kg, and two males each were dosed with 1000, 100, 10, and 1 mg/kg. Mortality occurred in all animals within 2 hours following dose administration at 5,000 mg/kg. Within 2 hours of lower doses, prostration was observed in male mice dosed at 1,000 mg/kg and lethargy was observed in male mice dosed at 100 mg/kg. The animals dosed at $\leq 1,000$ mg/kg appeared normal 24 hours after dose administration and throughout the observation period.

DICAMBA AMINE SALTS

Micronucleus Assay (84-2)

C. Toxicity Study

Groups of five male and five female mice were dosed with the DMA salt of dicamba by IP injection at 1,400, 2,000, 2,700, or 3,800 mg/kg. Mortality and body weight data for the toxicity study are presented in Appendix 1 (Table 1; study report page 14) included in this DER. Mortality occurred within 3 days in all animals dosed at 3,800 mg/kg, all males and 4/5 females dosed at 2,700 mg/kg, and in 2/5 females at 2,000 mg/kg. There were no deaths in animals dosed at 1,400 mg/kg. Clinical signs, first noted on the day of administration, included lethargy. LD_{50/3} was calculated by probit analysis to be 2,263 mg/kg. Based on these results, a high dose of 1,800 mg/kg (~80% of the LD_{50/3}) was chosen for the micronucleus assay.

D. Micronucleus Assay

1. Animal observations: Mortality was observed in 4/20 male and 3/20 female mice dosed at 1,800 mg/kg and in 1/15 males dosed at 900 mg/kg. Due to mortality in male mice treated at 900 mg/kg, only four males were available for analysis at the 24-hour sacrifice. Lethargy was observed in male and female mice at all dose levels.
2. Micronucleus assay: The results of the bone marrow micronucleus study are presented in Appendix 2 (Table 2; study report page 15) included in this DER. The DMA salt of dicamba was neither cytotoxic to the target organ nor caused a significant increase in micronucleated PCEs, compared with vehicle controls, in bone marrow cells collected from male or female mice 24, 48, or 72 hours after dosing with 450, 900, or 1,800 mg/kg. The positive control (40 mg/kg cyclophosphamide) induced significant ($p \leq 0.05$) increases in micronucleated PCEs in both sexes.

The study authors concluded that the DMA salt of dicamba was negative in this *in vivo* mouse micronucleus assay.

III. DISCUSSION/CONCLUSIONS

A. Investigator's Conclusions

The study authors concluded that the DMA salt of dicamba did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in mouse bone marrow, and was negative in the micronucleus test using male and female ICR mice.

B. Reviewer's Discussion

We agree with the study authors that the DMA salt of dicamba was negative in this *in vivo* micronucleus assay when tested to a dose level of 1,800 mg/kg. The sensitivity of this test to detect genotoxic response was demonstrated by the significant ($p \leq 0.05$) increase in micronucleated PCEs induced by the positive control (40 mg/kg CP). We conclude that the DMA salt of dicamba was adequately tested and found non-genotoxic in this *in vivo* micronucleus assay.

IV. STUDY DEFICIENCIES

No deficiencies were noted in this study.

APPENDIX 1

TABLE 1
TOXICITY STUDY WITH DMA SALT OF DICAMBA IN ICR MICE
BODY WEIGHT AND MORTALITY DATA

TREATMENT	SEX	GROUP MEAN BODY WEIGHTS (gms)			% CHANGE ¹		MORTALITY ²
		PRETREATMENT	DAY 1	DAY 3	DAY 1	DAY 3	
DMA Salt of Dicamba 1400 mg/kg	M	32.0 ± 1.2	31.1 ± 1.6	32.6 ± 1.3	-2.8%	1.9%	0 / 5
	F	23.3 ± 0.7	22.9 ± 0.6	24.4 ± 1.2	-1.7%	4.7%	0 / 5
2000 mg/kg	M	32.7 ± 1.6	31.0 ± 2.7	31.4 ± 4.4	-5.2%	-4.0%	0 / 5
	F	24.3 ± 0.8	22.0 ± 0.9	24.2 ± 0.7	-9.5%	-0.4%	2 / 5
2700 mg/kg	M	31.8 ± 1.3	NA ³	NA	NA	NA	5 / 5
	F	24.2 ± 1.6	21.3 ± 1.6	ND ⁴	-12.0%	ND	4 / 5
3800 mg/kg	M	32.4 ± 1.6	NA	NA	NA	NA	5 / 5
	F	24.2 ± 1.4	NA	NA	NA	NA	5 / 5

¹% Change = $\frac{(\text{Post-treatment weight} - \text{Pretreatment weight}) \times 100}{\text{Pretreatment weight}}$

²Reported as number of animals dead 3 days after dose administration/total number tested.

³NA = No data due to mortality.

⁴ND = Not determined due to weight of single surviving mouse was not measured.

APPENDIX 2

TABLE 2

SUMMARY OF BONE MARROW MICRONUCLEUS STUDY WITH DMA SALT OF DICAMBA IN ICR MICE

TREATMENT	SEX	TIME (HR)	NUMBER OF MICE	PCE/TOTAL ERYTHROCYTES	MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES	
					NUMBER PER 1000 PCE'S (MEAN \pm S.D.)	NUMBER PER PCE'S SCORED ¹
Water 10 ml/kg	M	24	5	0.64	0.8 \pm 1.79	4 / 5000
		48	5	0.57	1.2 \pm 0.84	6 / 5000
		72	5	0.61	0.0 \pm 0.00	0 / 5000
	F	24	5	0.57	0.4 \pm 0.55	2 / 5000
		48	5	0.64	0.6 \pm 0.89	3 / 5000
		72	5	0.48	0.0 \pm 0.00	0 / 5000
DMA Salt of Dicamba 450 mg/kg	M	24	5	0.77	1.0 \pm 1.00	5 / 5000
		48	5	0.60	0.4 \pm 0.55	2 / 5000
		72	5	0.59	0.4 \pm 0.55	2 / 5000
	F	24	5	0.53	0.6 \pm 1.34	3 / 5000
		48	5	0.60	0.2 \pm 0.45	1 / 5000
		72	5	0.53	0.0 \pm 0.00	0 / 5000
900 mg/kg	M	24	4	0.71	1.3 \pm 0.50	5 / 4000
		48	5	0.54	0.2 \pm 0.45	1 / 5000
		72	5	0.60	0.2 \pm 0.45	1 / 5000
	F	24	5	0.52	0.8 \pm 0.84	4 / 5000
		48	5	0.61	0.0 \pm 0.00	0 / 5000
		72	5	0.53	0.2 \pm 0.45	1 / 5000
1800 mg/kg	M	24	5	0.72	1.4 \pm 1.14	7 / 5000
		48	5	0.51	1.0 \pm 1.00	5 / 5000
		72	5	0.63	0.2 \pm 0.45	1 / 5000
	F	24	5	0.46	1.4 \pm 1.52	7 / 5000
		48	5	0.53	0.2 \pm 0.45	1 / 5000
		72	5	0.61	0.4 \pm 0.89	2 / 5000
CP, 40 mg/kg	M	24	5	0.41	9.4 \pm 6.91	47 / 5000*
	F	24	5	0.54	16.8 \pm 5.26	84 / 5000*

* $p \leq 0.05$ (Kastenbaum-Bowman Tables)

DATA EVALUATION RECORD**DICAMBA AMINE SALTS**

Study Type: 84-2; Micronucleus Assay in Mice (DMA Salt)

Work Assignment No. 1-14A (MRID 43354332)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer:

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Date: 4/12/96

Secondary Reviewer:

Steven Brecher, Ph.D.Signature: Steven Brecher
Date: 4/15/96

Project Manager:

William Spangler, Ph.D.Signature: William Spangler
Date: 4/8/96

Quality Assurance:

Reto Engler, Ph.D.Signature: Reto Engler
Date: 4/8/96**Disclaimer**

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

DATA EVALUATION RECORD

DICAMBA AMINE SALTS

Study Type: 84-2; Micronucleus Assay in Mice (DGA Salt)

Work Assignment No. 1-14B (MRID 43354333)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
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Signature: Mary Menetrez
Date: 4/17/96

Secondary Reviewer:
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Signature: Steven Brecher
Date: 4/15/96

Project Manager:
William Spangler, Ph.D.

Signature: William J. Spangler
Date: 4/18/96

Quality Assurance:
Reto Engler, Ph.D.

Signature: Reto Engler
Date: 4/18/96

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

DICAMBA AMINE SALTS

Micronucleus Assay (84-2)

EPA Reviewer: Jess Rowland, M.S. *Jess Rowland 6/20/97*
Branch Senior Scientist, Science Analysis Branch

EPA Secondary Reviewer: Alberto Protzel, Ph.D. *Alberto Protzel 7/29/98*
Branch Senior Scientist, Toxicology Branch I

DATA EVALUATION RECORD

STUDY TYPE: *In vivo* mammalian cytogenetics - micronucleus assay in mice

OPP Guideline Number: §84-2

DP BARCODE: D207648

SUBMISSION CODE: None

P.C. CODE: 128931

TOX. CHEM. NO.: 295F

TEST MATERIAL (PURITY): Diglycolamine (DGA) salt of dicamba (39.7% ai)

SYNONYMS: DGA salt of dicamba

CITATION: Putman, D., and R. Young. (1994) Micronucleus cytogenetic assay in mice. Microbiological Associates, Bethesda, MD. Laboratory Study Number TE237.122. 8/5/94. **MRID No.43354333**. Unpublished.

SPONSOR: Sandoz Agro, Inc., Des Plaines, IL.

EXECUTIVE SUMMARY: In an *in vivo* mouse bone marrow micronucleus assay (MRID No. 43354333), groups of five ICR mice/sex received a single IP injection of 525, 1050, or 2100 mg/kg of the DGA salt formulation of dicamba (39.7% ai). Bone marrow cells were harvested at 24, 48, or 72 hours post treatment and scored for micronucleated polychromatic erythrocytes (MPCEs).

Mortality occurred in 3/20 male and 1/20 female mice dosed at 2100 mg/kg. Lethargy was observed in male and female mice at all dose levels. Cytotoxicity by the DGA salt formulation was observed by a reduction in the ratio of PCEs to total erythrocytes in males dosed at 2100 mg/kg 48 and 72 hours following dosing. The positive control induced significant increases in MPCEs in both sexes. **The DGA salt of dicamba was non-mutagenic. There was no significant increase in the frequency of MPCEs in bone marrow after any treatment time.**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirements for *in vivo* cytogenetic mutagenicity data (§84-2).

DICAMBA AMINE SALTS

Micronucleus Assay (84-2)

I. MATERIALS AND METHODS

A. MATERIALS1. Test Material: DGA salt of dicamba

Description: Caramel-colored viscous liquid

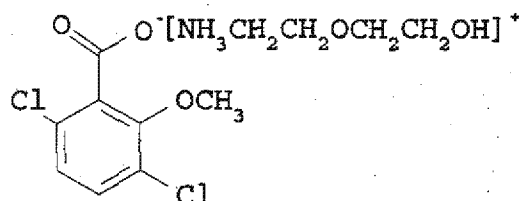
Lot/Batch #: 5998-1

Purity: 39.7% ai

Stability of compound: Not reported

CAS #: 104040-79-1

Structure:



Solvent used: Deionized distilled water

Other comments: The test material was stored at room temperature and protected from light.

2. Control Materials

Vehicle/Final volume/Route of administration: Deionized distilled water, 10 mL/kg, IP injection

Positive/Final dose(s)/Route of administration: Cyclophosphamide (CP) in deionized distilled water; 40 mg/kg

3. Test compound administration

Volume of test substance administered: 10 mL/kg

Route of administration: IP injection

Dose levels used:

Pilot Study: 1, 10, 100, 1000, 5000 mg/kg

Toxicity Study: 1400, 2000, 2700, 3800 mg/kg

Micronucleus Assay: 525, 1050, 2100 mg/kg

Rationale for dose selection: The high dose of 2100 mg/kg was approximately 80% of the LD_{50/3} determined from the toxicity study.

4. Test animals

a. Species: Mouse (Strain ICR)

Age: 6-8 weeks

Weight: Pilot Study, male 29.1-35.2 g, female 22.2-25.6 g; Toxicity Study, male 29.7-33.6 g, female 22.4-25.3 g; Micronucleus Assay, male 29.5-36.6 g, female 25.5-32.0 g

Source: Harlan Sprague Dawley, Inc., Frederick, MD.

b. No. animals used per dose:

Pilot Study: 5/sex at 5,000 mg/kg, and 2 males/dose at 1, 10, 100, or 1000 mg/kg

Toxicity Study: 5/sex/dose

Micronucleus Assay: 15/sex/dose, plus 5/sex as replacement animals at the high dose. An additional 5/sex received the positive control substance.

c. Properly maintained? Yes

B. TEST PERFORMANCE1. Treatment and Sampling Times

a. Test compound and vehicle control:

Dosing: Once

Sampling: 24, 48, and 72 hours after dosing

b. Positive control:

Dosing: Once

Sampling: 24, 48, and 72 hours after dosing

2. Tissues and Cells Examined

Bone marrow was the only tissue examined.

No. of polychromatic erythrocytes (PCEs) examined per animal: 1,000No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal: 1,000 erythrocytes were counted and the proportion of PCEs to total erythrocytes was calculated.3. Details of slide preparation

At 24, 48, and 72 hours after dosing, animals from each dose group were sacrificed by CO₂ asphyxiation. Marrow was aspirated from the femur and mixed with fetal bovine serum. After centrifugation and resuspension, cells were spread on slides, fixed in methanol, stained with May-Grunwald-Giemsa, and permanently mounted. Slides were coded prior to scoring.

4. Statistical methods

The incidence of MPCEs per 1,000 PCEs was determined for each animal and treatment group. Statistical significance ($p \leq 0.05$) of the incidence of MPCEs was determined using Kastenbaum-Bowman tables.

5. Evaluation Criteria

The test was considered valid if the number of MPCEs in the negative (vehicle) control did not exceed 5/1,000 PCEs and if the incidence of MPCEs in the positive control significantly increased with respect to the negative control ($p \leq 0.05$).

A positive response was a dose-responsive increase in the MPCEs with one or more dose levels statistically elevated relative to the vehicle control ($p \leq 0.05$, Kastenbaum-Bowman tables).

- C. COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

II. REPORTED RESULTS

A. Solubility/Analytical Determinations

The test material was soluble in water. The dosing solutions were prepared on the days of testing and samples were analyzed by HPLC to confirm the nominal concentrations. The dosing solutions were 99-103% of the nominal concentrations.

B. Pilot study

In a pilot study, five mice/sex were administered the DGA salt of dicamba by IP injection at 5000 mg/kg and to two male mice each at 1, 10, 100, or 1000 mg/kg. Within 2 hours of dosing at 5000 mg/kg, mortality occurred in 5/5 males and 5/5 females. Also within 2 hours of dosing, lethargy was observed in mice dosed at 1000 mg/kg. The mice dosed at 1000 mg/kg appeared normal within 72 hours of dosing and other animals dosed at ≤ 100 mg/kg appeared normal throughout the observation period.

DICAMBA AMINE SALTS

Micronucleus Assay (84-2)

C. Toxicity Study

Groups of five mice/sex were dosed with the DGA salt of dicamba by IP injection at 1400, 2000, 2700, or 3800 mg/kg. Body weight and mortality data for the toxicity study are presented in Appendix 1 (Table 1; study report page 14) included in this DER. Mortality occurred within 3 days in all animals dosed at 3800 mg/kg, in 2/5 males dosed at 2700 mg/kg, and in 1/5 males dosed at 2000 mg/kg. There were no deaths in animals dosed at 1400 mg/kg or females dosed at 2000 or 2700 mg/kg. Clinical signs, first noted on the day of dose administration, included lethargy. $LD_{50/3}$ was calculated by probit analysis to be approximately 2612 mg/kg. Based on these results, a high dose of 2100 mg/kg (approximately 80% of the $LD_{50/3}$) was chosen for the micronucleus assay.

D. Micronucleus Assay

Separate studies were performed for male and female mice.

1. Animal observations: Mortality was observed in 3/20 male and 1/20 female mice dosed at 2100 mg/kg. Lethargy was observed in male and female mice at all dose levels.
2. Micronucleus assay: The results of the bone marrow micronucleus assay are presented in Appendix 2 (Table 2; study report page 15) included in this DER. The DGA salt of dicamba was cytotoxic to the bone marrow; a reduction in the ratio of PCEs to total erythrocytes was observed in male mice 48 and 72 hours following dosing at 2100 mg/kg. The DGA salt formulation of dicamba did not cause a statistically significant increase in MPCEs compared to vehicle controls in bone marrow cells collected from male or female mice 24, 48, or 72 hours after dosing at 525, 1050, or 2100 mg/kg. The positive control (40 mg/kg cyclophosphamide) induced significant ($p \leq 0.05$) increases in MPCEs in both sexes.

The study authors concluded that the DGA salt formulation of dicamba was negative in this *in vivo* mouse micronucleus assay.

III. DISCUSSION/CONCLUSIONS

A. Investigator's Conclusions

The study authors concluded that the DGA salt formulation of dicamba did not induce a significant increase in the incidence of micronucleated polychromatic erythrocytes in mouse bone marrow, and was negative in the micronucleus test using male and female ICR mice.

B. Reviewer's Discussion

The reviewer agrees with the study authors that the DGA salt formulation of dicamba was not clastogenic or aneugenic in this *in vivo* assay when tested to a dose level of 2100 mg/kg. The sensitivity of this test to detect genotoxic response was demonstrated by the presence of pharmacotoxic effects and the significant ($p \leq 0.05$) increase in MPCEs induced by the positive control (40 mg/kg CP). We conclude that the DGA salt of dicamba was adequately tested and found non-genotoxic in this *in vivo* micronucleus assay.

IV. STUDY DEFICIENCIES

None

APPENDIX 1

TABLE 1
TOXICITY STUDY WITH DGA SALT OF DICAMBA IN ICR MICE
BODY WEIGHT AND MORTALITY DATA

TREATMENT	SEX	GROUP MEAN BODY WEIGHTS (gms)			% CHANGE ¹		MORTALITY ²
		PRETREATMENT	DAY 1	DAY 3	DAY 1	DAY 3	
DGA Salt of Dicamba 1400 mg/kg	M	32.4 ± 1.5	31.8 ± 1.6	33.0 ± 1.8	-1.9%	1.9%	0 / 5
	F	23.5 ± 1.0	23.0 ± 1.2	23.1 ± 0.8	-2.1%	-1.7%	0 / 5
2000 mg/kg	M	30.6 ± 2.2	30.6 ± 1.5	31.4 ± 0.9	0.0%	2.6%	1 / 5
	F	23.6 ± 0.6	23.0 ± 1.0	24.3 ± 0.9	-2.5%	3.0%	0 / 5
2700 mg/kg	M	31.5 ± 1.1	29.4 ± 3.0	30.5 ± 4.1	-6.7%	-3.2%	2 / 5
	F	24.2 ± 1.6	22.1 ± 1.4	24.8 ± 1.3	-8.7%	2.5%	0 / 5
3800 mg/kg	M	31.7 ± 1.6	NA ³	NA	NA	NA	5 / 5
	F	20.6 ± 0.9	NA	NA	NA	NA	5 / 5

¹% Change = $\frac{(\text{Post-treatment weight} - \text{Pretreatment weight}) \times 100}{\text{Pretreatment weight}}$

²Reported as number of animals dead 3 days after dose administration/total number tested.

³NA = No data due to mortality.

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APPENDIX 2

TABLE 2
SUMMARY OF BONE MARROW MICRONUCLEUS STUDY WITH DGA SALT OF DICAMBA IN ICR MICE

TREATMENT	SEX	TIME (HR)	NUMBER OF MICE	PCE/TOTAL ERYTHROCYTES	MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES	
					NUMBER PER 1000 PCE'S (MEAN \pm S.D.)	NUMBER PER PCE'S SCORED ¹
Water 10 ml/kg	M	24	5	0.64	0.8 \pm 1.79	4 / 5000
		48	5	0.57	1.2 \pm 0.84	6 / 5000
		72	5	0.61	0.0 \pm 0.00	0 / 5000
	F	24	5	0.57	0.4 \pm 0.55	2 / 5000
		48	5	0.64	0.6 \pm 0.89	3 / 5000
		72	5	0.48	0.0 \pm 0.00	0 / 5000
DGA Salt of Dicamba 525 mg/kg	M	24	5	0.73	1.2 \pm 0.84	6 / 5000
		48	5	0.56	0.8 \pm 0.84	4 / 5000
		72	5	0.61	0.2 \pm 0.45	1 / 5000
	F	24	5	0.53	0.8 \pm 1.30	4 / 5000
		48	5	0.62	0.2 \pm 0.45	1 / 5000
		72	5	0.58	0.2 \pm 0.45	1 / 5000
1050 mg/kg	M	24	5	0.68	1.2 \pm 1.10	6 / 5000
		48	5	0.62	1.0 \pm 1.41	5 / 5000
		72	5	0.60	0.6 \pm 0.55	3 / 5000
	F	24	5	0.54	1.0 \pm 1.41	5 / 5000
		48	5	0.63	0.2 \pm 0.45	1 / 5000
		72	5	0.50	0.0 \pm 0.00	0 / 5000
2100 mg/kg	M	24	5	0.56	0.4 \pm 0.55	2 / 5000
		48	5	0.32	1.6 \pm 1.34	8 / 5000
		72	5	0.48	0.2 \pm 0.45	1 / 5000
	F	24	5	0.52	0.2 \pm 0.45	1 / 5000
		48	5	0.60	1.2 \pm 1.10	6 / 5000
		72	5	0.60	0.2 \pm 0.45	1 / 5000
CP, 40 mg/kg	M	24	5	0.41	9.4 \pm 6.91	47 / 5000*
	F	24	5	0.54	16.8 \pm 5.26	84 / 5000*

* $p \leq 0.05$ (Kastenbaum-Bowman Tables)

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Chemical:	Dicamba, dimethylamine salt; Dicamba, diglycoamine salt; Dicamba, isopropylamine salt
PC Code:	029802; 128931; 128944
HED File Code	13000 Tox Reviews
Memo Date:	08/12/1997
File ID:	TX012293
Accession Number:	412-01-0120

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02/09/2001